REMARKS

Entry of the foregoing, reexamination and further and favorable reconsideration of the subject application in light of the following remarks, are respectfully requested.

By the present amendment, independent Claims 46 and 52 have been amended. Support for the amendment of Claims 46 and 52 may be found, at the very least, in Examples 2-3, at pages 18-19, particularly at page 18, 18-21, and page 19, lines 23-25. No new matter is thus being added by this amendment.

Applicant respectfully requests a personal interview after the Examiner's initial review of this amendment, but prior to issuance of a further Official Action.

Prior to addressing the merits of the Examiner's rejection, a brief description of Applicant's invention is believed to be necessary. Essentially, the present invention is based on the surprising discovery that the use of pH gradients, in accordance with the invention, to load liposomes allows for the rapid uptake of drugs or chemical species by the liposomes. In addition, the chemical-species loaded liposomes of the invention are stable in the presence of the pre-imposed pH gradient and in the absence of the pre-imposed pH gradient. The claims, as amended, require that the stability of the liposomes is "independent of maintenance of a pH gradient across the liposome membrane after entrapment of the chemical species." This unexpected feature allows the liposomes to remain stably loaded after they are injected into a host where the pre-imposed pH gradient no longer exists. This feature of the present invention is important for preventing massive amounts of drug from leaking or being "dumped" in the host after administration of the drug loaded liposomes. Moreover, it allows

for the safe administration to animals, because the lack of leakage causes the animal to suffer "no long-term effects of the administration" (as recited by amended Claims 46 and 52).

Applicant has experimentally shown that it is not necessary to maintain the pH gradient to keep the liposomes in a loaded state. Example 3 of the present application demonstrates that when the liposomes were placed in a ten-fold excess of lysine buffer, the pH gradient that had been preimposed for loading was largely collapsed, and as a result, very little leakage of the entrapped chemical occurred. Moreover, Example 2 of the present application demonstrates that animals suffer no long-term effects of the liposome infusion.

The Examiner has rejected Claims 46-54, 56-57 and 61-64 under 35 U.S.C. §102(b) as being allegedly anticipated by Nichols. This rejection is respectfully traversed.

In contrast to the present invention, Nichols et al. teaches the uptake of catecholamine by liposomes maintaining a pH gradient. The liposomes of Nichols et al. remain loaded only in the presence of a pre-imposed pH gradient which suggests that upon administration to a host, where the pre-imposed pH gradient no longer exists, massive amounts of catecholamine would leak from the liposomes into the host. In fact, Nichols et al. stated that "[w]hen the gradients were destroyed by ammonium chloride additions, the accumulated catecholamines were released, demonstrating that the uptake was reversible and dependent upon pH gradients." Moreover, Nichols et al. does not disclose administration to a host, and therefore does not teach that the liposome preparation can be administered to animals with no long-term effects.

Therefore, based on the foregoing, withdrawal of the § 102 rejection based on Nichols et al. is respectfully requested.

The Examiner rejected Claims 46-54, 57 and 61-64 35 U.S.C. § 102(b) as allegedly being anticipated by Deamer et al. This rejection is respectfully traversed.

The primary focus of Deamer et al. is to analyze the effects of a pH gradient on flourescent probes. Deamer et al. only uses liposomes to analyze the quenching effect on flourescent probes in the presence a pH gradient. Deamer et al. does not analyze loading liposomes with chemical species to form stable liposome vesicle-entrapped chemical-species, and does not teach that the stability of the liposome is "independent of maintenance of a pH gradient across the liposome membrane after entrapment of the chemical species," or that this stability allows the animal to suffer "no long-term effects of the administration" (as recited by amended Claims 46 and 52).

Therefore, withdrawal of this rejection is respectfully requested.

The Examiner rejected Claims 46-54, 59 and 61-64 under 35 U.S.C. § 102(b) as allegedly being anticipated by Cramer et al. or Kano et al. This rejection is respectfully traversed.

Cramer et al. also teaches the physical chemistry involved in using a pH gradient to load certain simple ionizable molecules (fumaric and maleic acid) into a liposome. Kano et al. teaches the use of trisodium 8-hydroxy-1,3,6-pyrene-trisulfonate, pyranine, as a probe for monitoring the pH in the interiors of negatively charged liposomes and at the outer surface of positively charged liposomes. Neither reference teaches or suggests the claimed invention of using a pH gradient to produce a stable liposome vesicle-entrapped chemical species, much less that the stability of the liposome is "independent of maintenance of a pH gradient across

the liposome membrane after entrapment of the chemical species," or that this stability allows the animal to suffer "no long-term effects of the administration" (as recited by amended Claims 46 and 52).

The liposomes of Cramer et al. remain loaded only in the presence of a pH gradient which suggests that upon administration to a host, massive amounts of catecholamine would leak from the liposomes into the host. In fact, Cramer et al. states on page 299 (last line) that "following a pH perturbation, an equilibrium condition, corresponding to zero net transport, should be reached where the internal and external H_2A activities are equal." In other words, if liposomes produced in the presence of a pre-imposed pH gradient are placed in an environment where the pre-imposed pH gradient no longer exists, massive amounts of H_2A would leak from the liposomes. Cramer et al. states in the last paragraph on page 300 that non-selective leakage of both the fumaric and maleic acid probably is the result of vesicle rupture in response to osmotic stress. These statements teach away from the present invention where drug entrapped liposomes remain stably loaded after they are injected into a host where the pre-imposed pH gradient used to load the liposomes no longer exists. Upon reading these references, the skilled artisan would not be lead to the present invention.

Withdrawal of this rejection is therefore respectfully requested.

Claims 46-64 have been rejected under 35 U.S.C. § 103(a) as allegedly being unpatentable over Nichols et al., Deamer et al., Cramer et al. or Kano et al. This rejection is respectfully traversed.

Applicant respectfully submits that the subject invention is not taught or suggested by any of the references cited by the Examiner. These references, taken alone or in combination, do not teach or suggest to one skilled in the art that the stability of the liposome is

"independent of maintenance of a pH gradient across the liposome membrane after entrapment of the chemical species," or that this stability allows the animal to suffer "no long-term effects of the administration" (as recited by amended Claims 46 and 52).

It was only with the present invention that liposomes which could be injected into a rat *in vivo* for the delivery of drugs (See Example 3 in the present application) were obtained and recognized as such. Prior to these experiments, one would not have known whether such drug entrapped liposomes would wreak havoc on the biogenic amines that play a vital role in animal physiology. For example, as demonstrated in Deamer et al., catecholamines could be loaded into liposomes with pH gradients. However, until after Applicant's *in vivo* experiments were performed, no one could have predicted that an animal would tolerate the injection of such catecholamine-loaded liposomes, because none of the cited references discloses or suggests liposomes which retain their stability in the absence of a pH gradient across the liposomal membrane.

In the Office Action dated November 16, 2000, the Examiner stated that Applicant's arguments distinguishing the prior art on the basis that it does not show that liposomes containing a therapeutic drug product would retain their contents upon administration to an animal were "not found to be persuasive since the differences argued are not reflected in the claims." (page 3 of the November 16, 2000 Office Action) In light of the present amendment, which amends the independent claims to explicitly recite that the stability of the liposome is "independent of maintenance of a pH gradient across the liposome membrane after entrapment of the chemical species," and that this stability allows the animal to suffer "no long-term effects of the administration", Applicants submit that withdrawal of the rejections is warranted, and is thus respectfully requested.

Further and favorable action in the form of a Notice of Allowance is respectfully requested.

In the event that there are any questions relating to this Amendment, or the application in general, prior to the requested personal interview, it would be appreciated if the Examiner would contact the undersigned attorney by telephone so that prosecution is expedited. In any event, the undersigned awaits being contacted for the requested personal interview.

Respectfully submitted,

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Attachment to Amendment and Reply dated October 1, 2002

Marked-up Claims 46 & 52

46. (Twice Amended) A method for preparing a stable liposome vesicle-entrapped chemical species which comprises the steps of:

(a) forming liposomes comprising a membrane in:

(1) an aqueous medium containing an acid which is substantially impermeable through the vesicle to give an acidic liposome-containing aqueous medium in which the acid is present in the internal and external liposome phases; or

(2) an aqueous medium containing a base which is substantially impermeable through the vesicle to give an basic liposome-containing aqueous medium in which the base is present in the internal and external liposome phases;

(b) adding:

(1) to the thus-obtained acid liposome-containing aqueous medium a permanently charged, chargeable, or pH titratable chemical species which is a cationic chemical species, or

(2) to the thus-obtained acid liposome-containing aqueous medium a permanently charged,

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Marked-up Claims 46 & 52

chargeable, or pH titratable chemical species which is an anionic chemical species; and

(c) adding to the external liposome phase:

(1) a base to provide a pH gradient across the membrane of the liposome and thereby induce the cationic chemical species to pass into the liposomes' internal acidic aqueous phase, or

(2) an acid to provide a pH gradient across the membrane of the liposome and thereby induce the anionic chemical species to pass into the liposomes' internal basic aqueous phase;

wherein said cationic chemical species or said anionic chemical species is accumulated and entrapped within said liposome to produce a stable liposome vesicle-entrapped chemical species, said stability being independent of maintenance of a pH gradient across the liposome membrane after entrapment of the chemical species such that after administration to an animal the chemical species is carried to its destination by the liposome vesicle before significant leakage occurs, and the animal suffers no long-term effects of the administration.

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Marked-up Claims 46 & 52

52. (Twice Amended) A method of preparing a stable liposome vesicle-entrapped chemical species, which method comprises:

(a) forming liposomes in:

(1) an aqueous medium containing an acid which is substantially impermeable through the vesicle to give an acidic liposome-containing aqueous medium in which the acid is present in the internal and external liposome phases; or

(2) an aqueous medium containing a base which is substantially impermeable through the vesicle to give an basic liposome-containing aqueous medium in which the base is present in the internal and external liposome phases;

(b) adding:

(1) to the thus-obtained acid liposome-containing aqueous medium a permanently charged, chargeable, or pH titratable chemical species which is a cationic chemical species, or

(2) to the thus-obtained acid liposome-containing aqueous medium a permanently charged,

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Marked-up Claims 46 & 52

chargeable, or pH titratable chemical species which is an anionic chemical species; and

(c) adding to the external liposome phase:

(1) a base in an amount effective to create a pH gradient between the external liposome phase and the internal liposome phase to thereby induce the cationic chemical species to pass into the liposomes' internal acidic aqueous phase, or

(2) an acid in an amount effective to create a pH gradient between the external liposome phase and the internal liposome phase to thereby induce the anionic chemical species to pass into the liposomes' internal basic aqueous phase;

wherein said cationic chemical species or said anionic chemical species is accumulated and entrapped within said liposome to produce a stable liposome vesicle-entrapped chemical species, said stability being independent of maintenance of the pH gradient after entrapment of the chemical species such that after administration to an animal the chemical species is carried to its destination by the liposome vesicle before significant leakage occurs, and the animal suffers no long-term effects of the administration.